

बाहर की चुनौतियों से नहीं हम अपने
अंदर की कमजोरियों से हारते हैं।

CSIR NET – Life Science

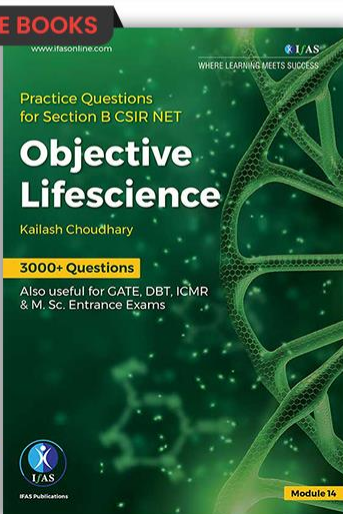
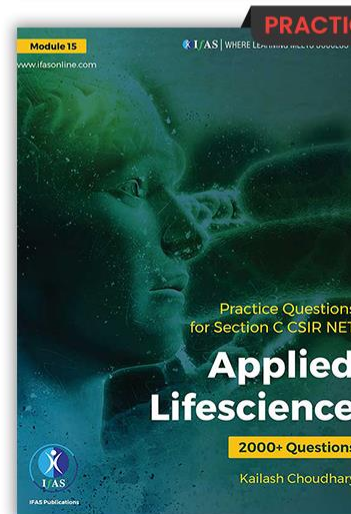
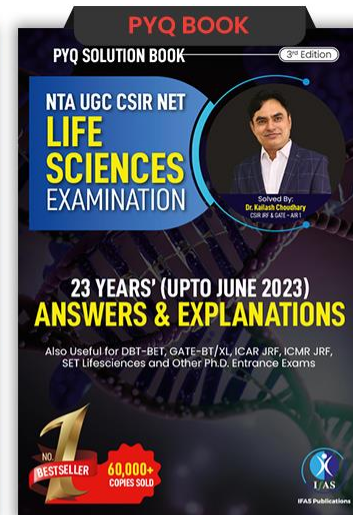
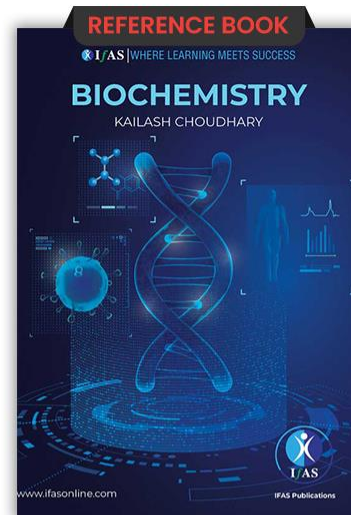
Unit 1: Biochemistry

09

Amino Acids











Order Online and Get
Free Delivery Across India





Points to be covered in this Lecture

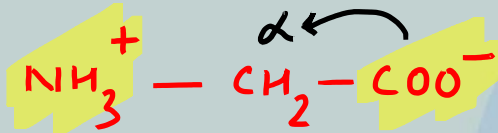
-  Standard Amino Acids
-  Basic Properties
-  Non-polar Amino Acids
-  Polar Amino Acids
-  pI of amino Acids and Peptides
-  Peptide Bond
-  Molecular weight of peptide
-  Charge on peptide





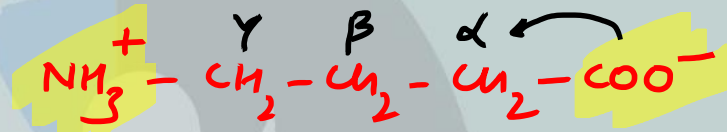
What are amino acids?

Organic molecules which contain both amino and carboxylic acid groups.



Glycine

[α -Amino acetic acid]



[γ -Amino Butyric Acid (GABA)]

Total Number of Amino acids?

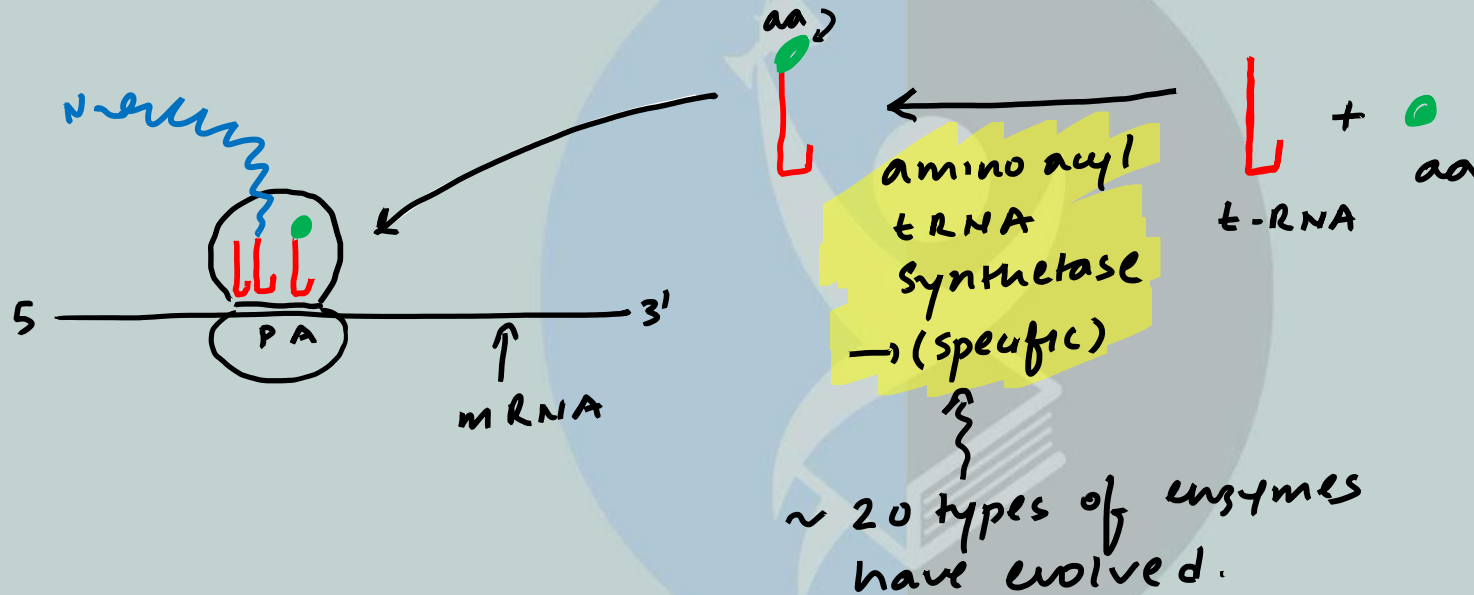
Over 500 amino acids exist in nature

- metabolic intermediate
- neurotransmitter
- other physiological function



Standard Amino Acids \rightarrow 20 + Selenocysteine₂₁ + Pyrrolysine₂₂

✓ Building blocks of proteins



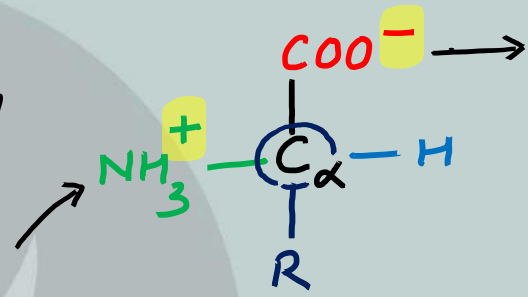
Important Properties of Standard Amino Acids

α -Amino Acid

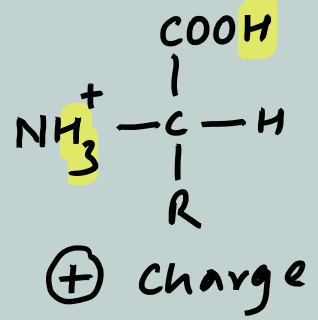
1. Zwitter ion

2. 2 or 3 pKa Values (ionizable group in side chain)

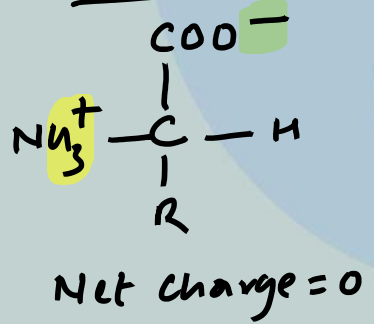
3. Charge depends on surrounding pH



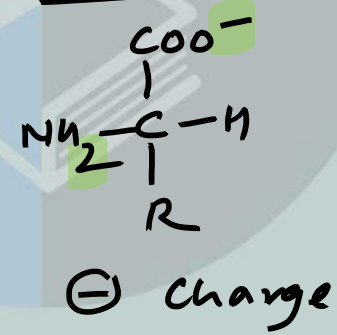
pH = 1



pH = 7



pH = 12



α -COOH $pK_{a1} = 2$
 α -NH₂ $pK_{a2} = 9.5$



Exist in L- form except glycine : Lack chiral carbon)

- most of aa has 1 chiral center
- Isoleucine & threonine has 2 chiral center
- Stereoisomers = 2^n

Exist in S- form except glycine and Cysteine (R-form)

Glycine = NO R or S- form

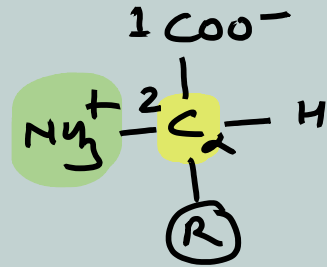
Cysteine = R- form

Rest of aa = C2 → S- form

Isoleucine C₂ : S & C₃ : S

Threonine C₂ : S & C₃ : R

Enantiomers





On basis of their side chain divided two groups- Non-polar and polar Amino Acids

Non-polar Amino Acids

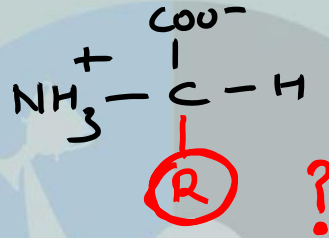
- Hydrophobic
- Seen in interior of protein

Aliphatic

Glycine, Alanine, valine,
Leucine, Isoleucine

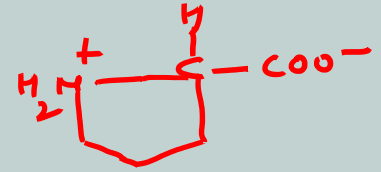
'GAVLI'

- methionine



Aromatic

Phenylalanine, Tryptophan



Cyclic

Proline



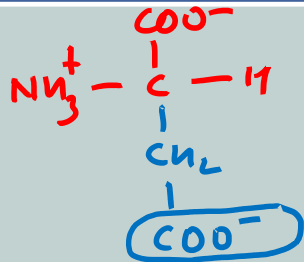
Polar Charged

pH = 7

\ominus

Acidic

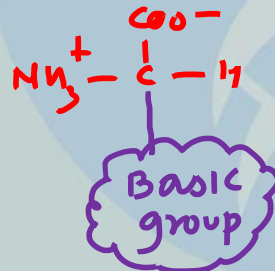
Aspartic Acid
Glutamic Acid



\oplus

Basic

Lysine
Arginine
Histidine



Polar Uncharged

Serine
Threonine
Tyrosine

— OH
Group

Asparagine
Glutamine

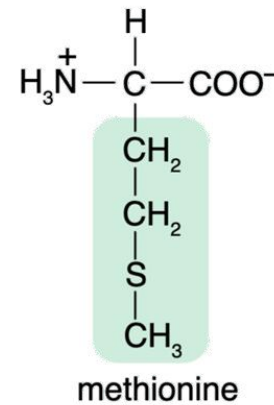
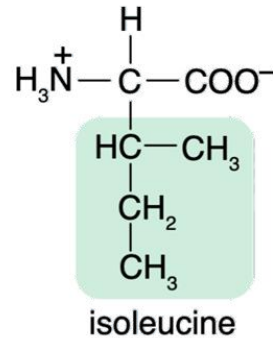
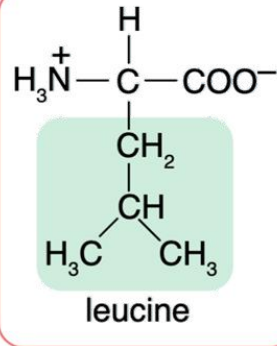
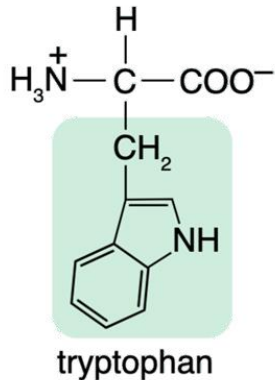
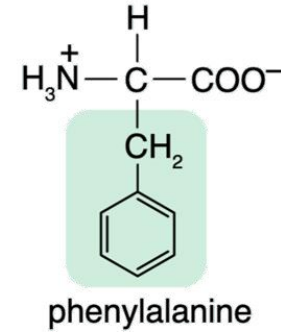
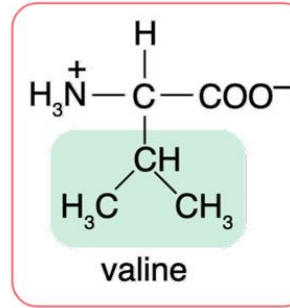
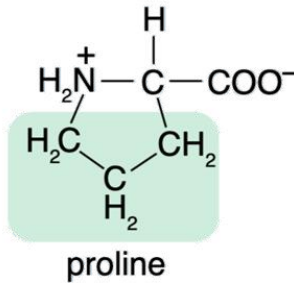
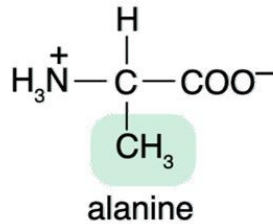
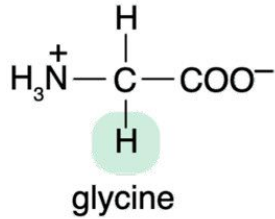
$\text{C}=\text{O} - \text{NH}_2$
Amide

Cysteine

SH group



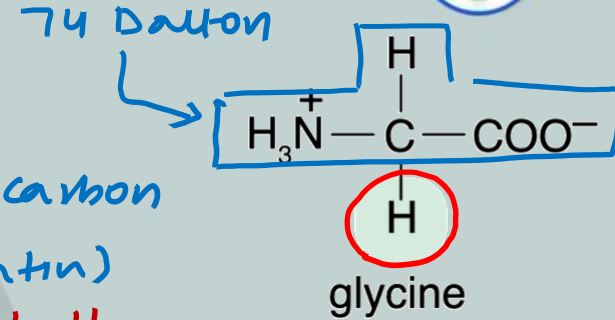
I. Amino acids with Non-Polar Aliphatic R-chain





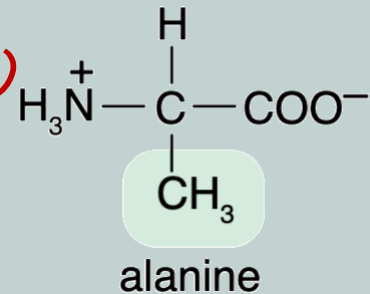
1. Glycine (G):

- ✓ Smallest amino acid $R = H$ $MW = 75$ Dalton
- ✓ **optically inactive**, NO stereoisomer, LACK chiral carbon
- ✓ Abundance in silk fibroin (40%), Collagen (33 %) (gelatin)
- ✓ Precursor for δ -amino levulinic acid (Heme) or chlorophyll
- ✓ Inhibitory neurotransmitter (ALA)
- ✓ Occupy Maximum area on Ramachandran plot (minimum steric hindrance)



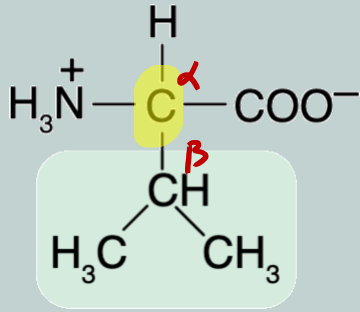
2. L-Alanine (A):

- Glucose-alanine cycle – N-transport form – muscle \rightarrow Liver
- D-Alanine in bacterial cell wall (trans peptide chain)

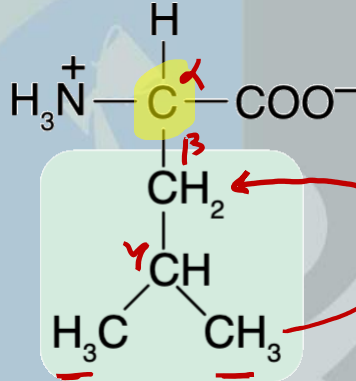


Branched Chain Amino acids

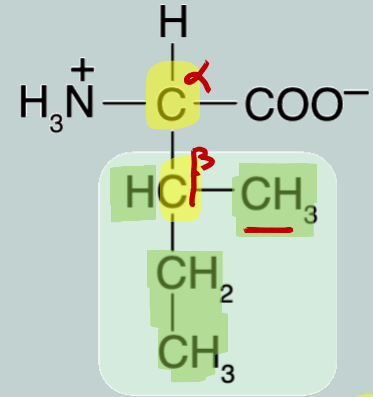
3. L- Valine (V)



4. L- Leucine (L)



5. L- Isoleucine (I)



→ Essential amino acid

If we cannot oxidize
branched chain aa



Autosomal Recessive
Disorder



Enzyme missing

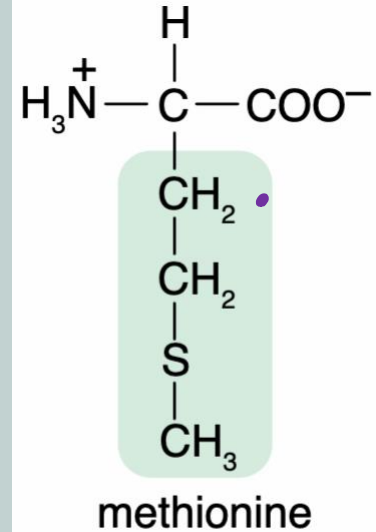
Two chiral carbon: 2S, 3S
= 4 stereoisomers

Maple syrup urine disease: Branched chain α-keto acid dehydrogenase.



6. L- Methionine (M):

- ✓ Methionine is an essential sulphur containing amino acid
- ✓ First amino acid
- ✓ S-adenosyl methionine (SAM) → precursor for ethylene
- ✓ Pulses (Legumes) lack in it. → methyl donor





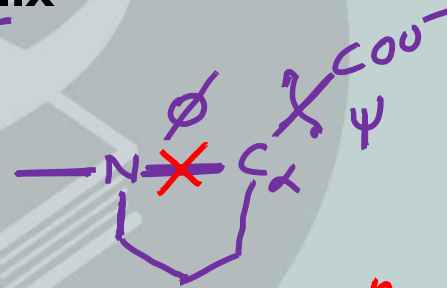
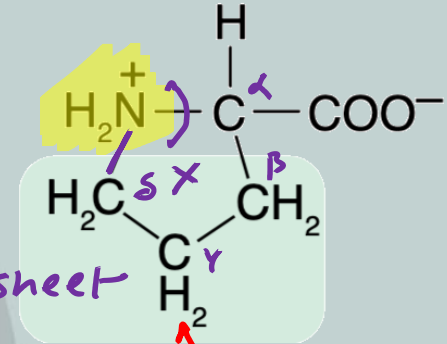
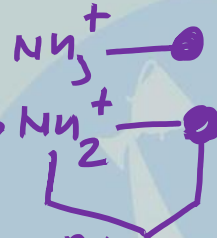
Cyclic aliphatic R-chain

7. Proline (P)

- **α -imino acid**
- **Structural disruptor** \rightarrow Absent in α -helix, β -sheet
- Present in β -turn and **polyproline helix**
- Least area on Ramachandran plot
- Compatible osmoytes
- Hydroxylation of proline

4-Hydroxy Proline
(Polar)

Restricted rotation betⁿ
N & C $_{\alpha}$ (ϕ)

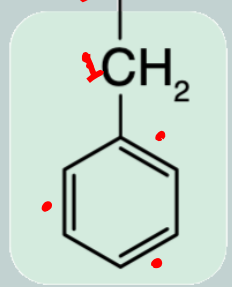
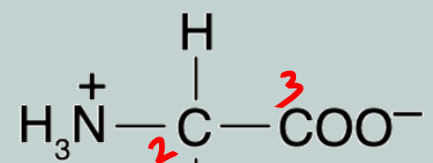


II. Amino acid with aromatic R chain.

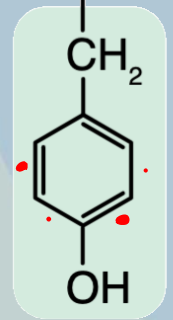
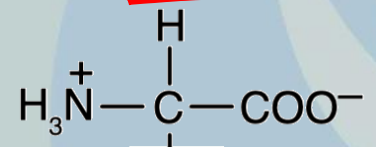
- Phenyl alanine, tyrosine and tryptophan.
- Produced by shikimic acid pathway in plants and bacteria
- Absorb UV light between 260-280 nm (Near UV)

Precursor : PEP +
Erythrose-4-P

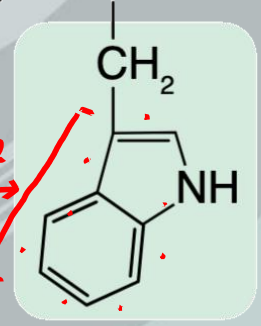
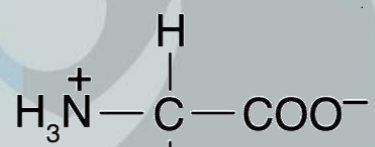
Enoyl Pyruvate
Shikimate Phosphate
Synthase (EPSPS)
enzyme



phenylalanine
258 nm



tyrosine
274 nm



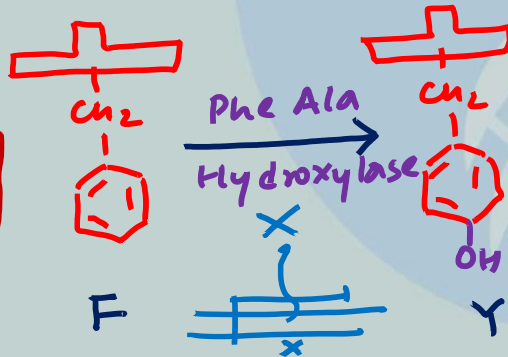
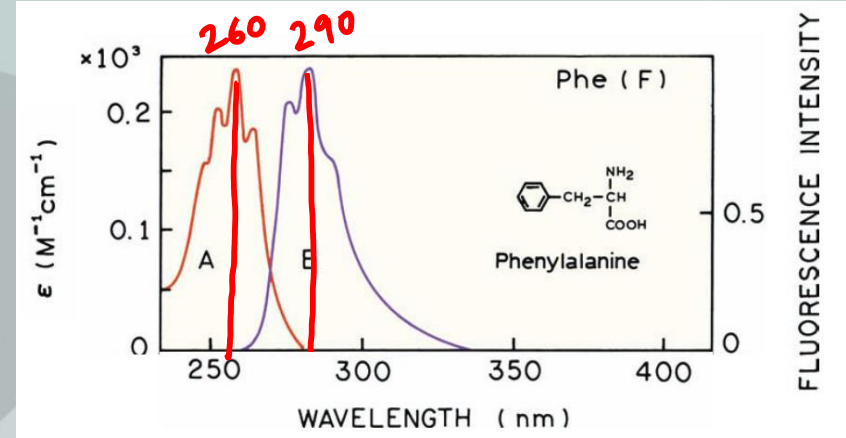
tryptophan
280 nm.

T[⊖]
Glyphosate
(Round Up)
Herbicide



8. Phenylalanine (F): Non-polar Aromatic

- ✓ Most hydrophobic
- Precursor for tyrosine and phenyl propanoids
- Essential amino acids ✓
- Maximum absorbance 260 nm ✓
- Emission at 290 nm ✓



Phenyl Keton
uria

9. Tyrosine (Y): Polar Aromatic

Precursor for

- L-DOPA, Dopamine
- Catecholamines (epinephrine)
- Thyroxine, Melanin
- Morphine.

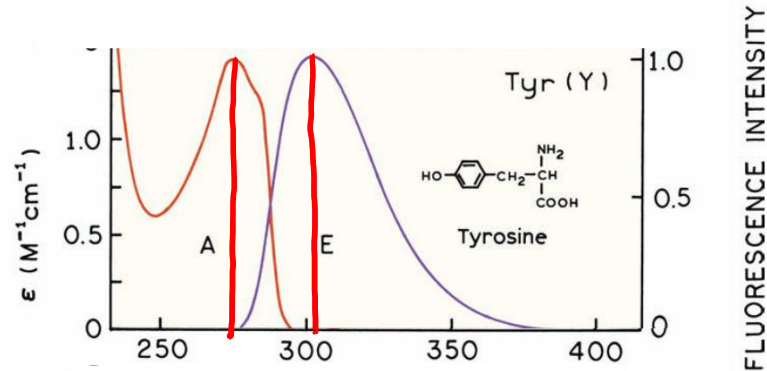
Phosphorylation

← Post translational modification (PTM)

Phenylalanine hydroxylase

Phe

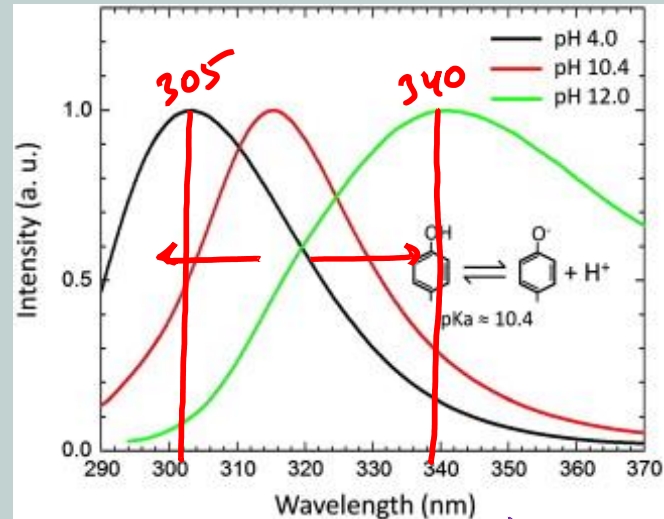
Tyr



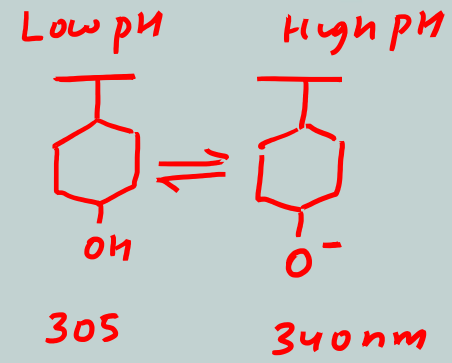
Absorption: 270 nm

Emission: 310 nm

Emission spectrum depends on surrounding pH



pH 4 = 305 nm
pH 10.4 = 315 nm
pH 12.0 = 340 nm



VIBGYOR

← Lower pH : Blue shift
 Higher pH : Red shift

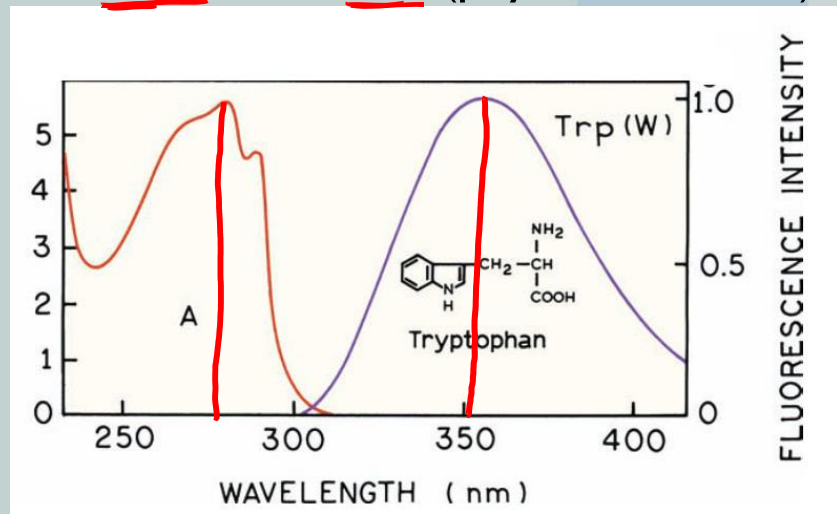
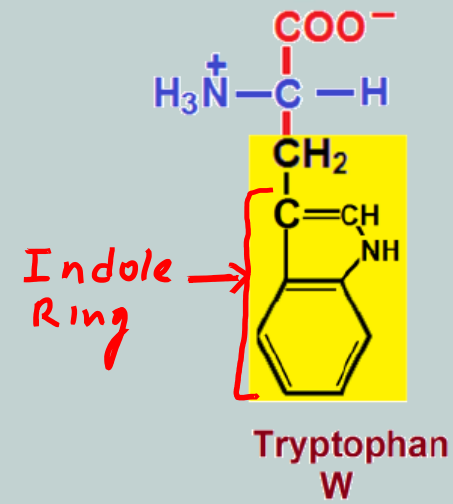
10. Tryptophan: Non-polar Aromatic (W)

Largest aa

mw = 204 D

Sudden Infant Death Syndrome

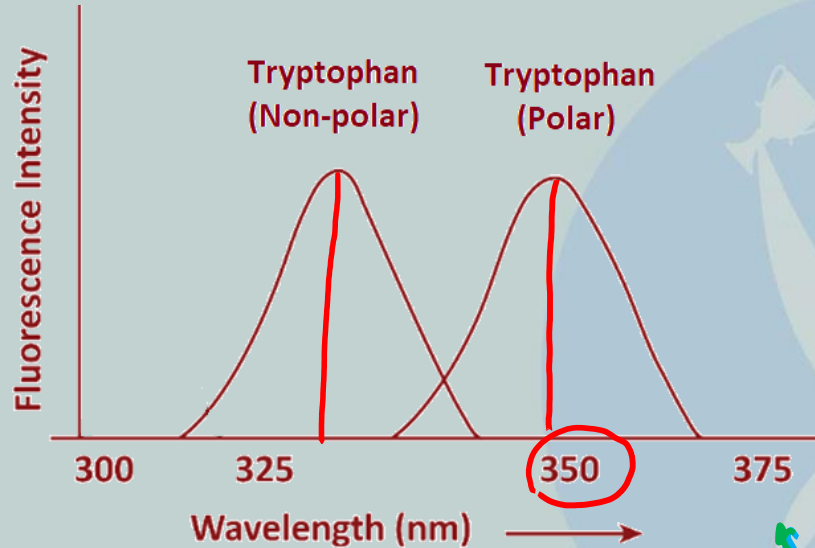
- ✓ Intrinsic fluorescent probe (environment)
- ✓ Serotonin (a neurotransmitter) ↑ - SIDS
- ✓ Melatonin (a neurohormone)
- ✓ Niacin, also known as vitamin B3
- ✓ Auxins like IAA (phytohormones)



Absorption: 280 nm

Emission: 350 nm

Tryptophan Emission Depend on Environment

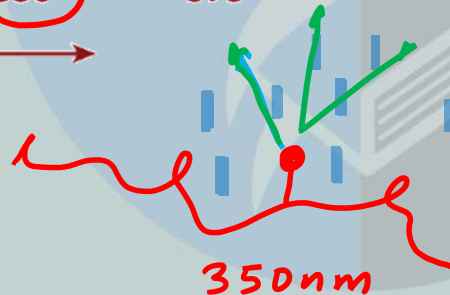
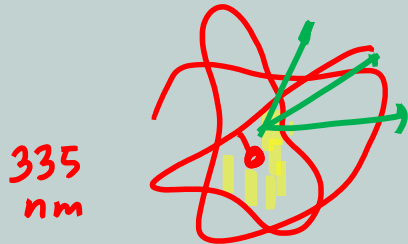


Folded Protein – Non-polar Envr.

- **335 nm**

Unfolded protein – Polar Envr.

- **350 nm**





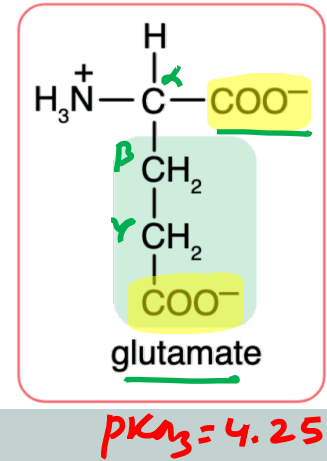
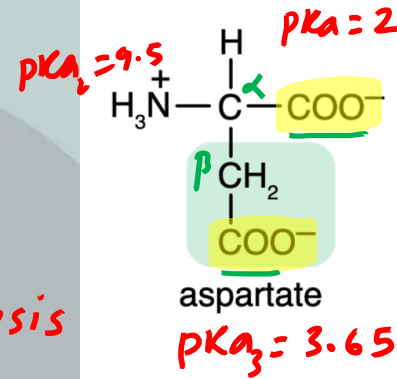
III. Polar -ve Charged - Acidic amino acids

I I. Aspartic Acid (D):

- ✓ Urea cycle
- ✓ Pyrimidine *and Purine de novo biosynthesis*
- ✓ Excitatory Neurotransmitter
- ✓ Phosphorylation (PTM)

✓ 12. Glutamic acid (E):

- ✓ Excitatory Neurotransmitter.
- ✓ Precursor for GABA (glutamate decarboxylase-GAD)
- ✓ Food additive and flavor enhancer (*Mono sodium Glutamate*)





IV. Polar + Charged: Basic amino acids

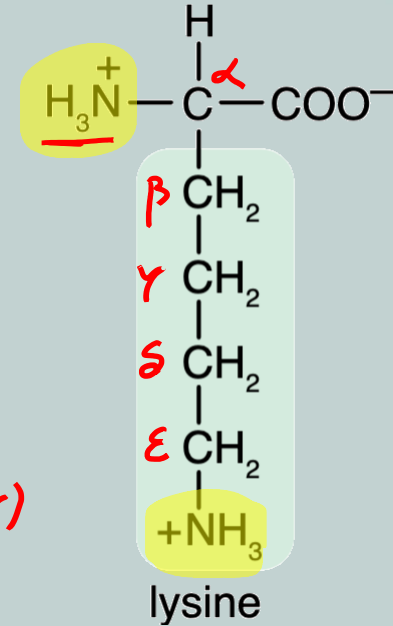
13. Lysine (K): $pK_a = 10.5$

- Cereals lack it. , *essential aa.*

- Post translational modifications like

- ✓ methylation,
- ✓ acetylation,
- ✓ sumoylation,
- ✓ ubiquitination,
- ✓ hydroxylation

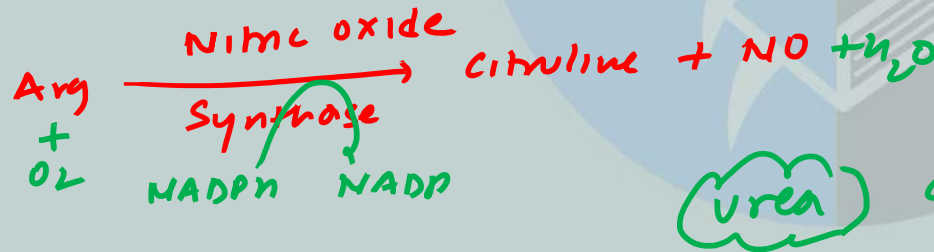
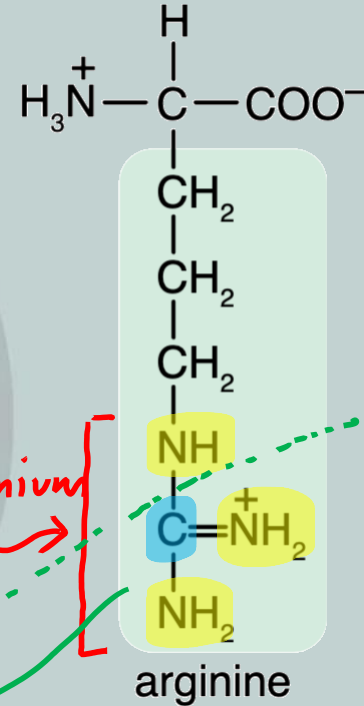
(small ubiquitin like modifier)
SUMO protein





14. Arginine (R)

- ✓ Most basic amino acid $pK_{a3} = 12.5$
- ✓ Abundant in histones and protamines (sperm)
- ✓ Guanidinium group
- ✓ Precursor for NO (Gaseous signal)
- ✓ Urea



Guanidinium Group

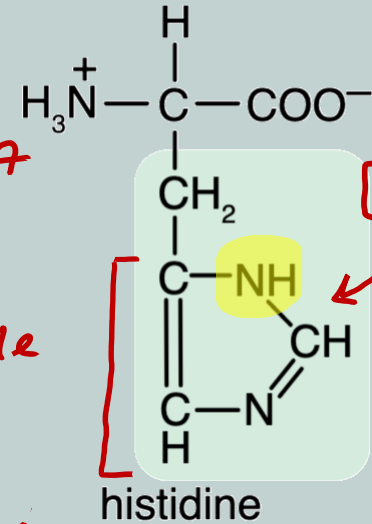


15. Histidine (H) *weak base*

- ✓ R group with pKa value of 6.0, *Role as buffer at pH 7*
- ✓ Active site
- ✓ Phosphorylation (PTM)
- ✓ Histamine
- ✓ High affinity for Nickel

*Imidazole
Ring
↓
Aromatic
Ring*

UV absorption = 235 nm

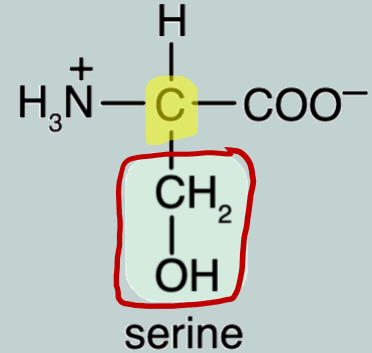




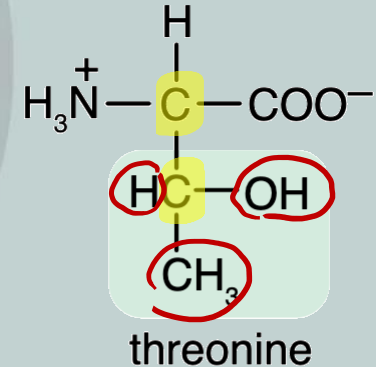
V. Polar uncharged amino acid with OH group

16. Serine (S)

- Catalytic function (nucleophilic activity)
- O-linked glycosylation (PTM)
- Phosphorylation



17. Threonine (T)



1. Two asymmetric carbon (2S, 3R)

✓ 2. O-linked glycosylation.

✓ 3. Phosphorylation



VI. Polar uncharged amino acid with amide group

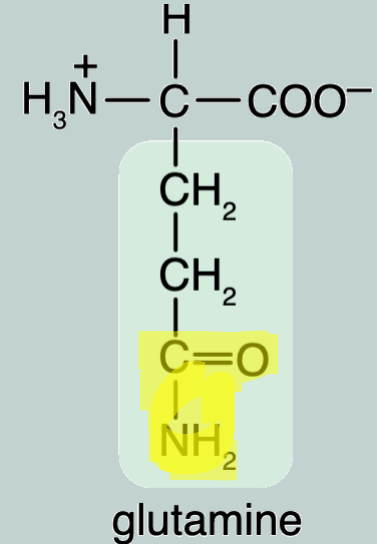
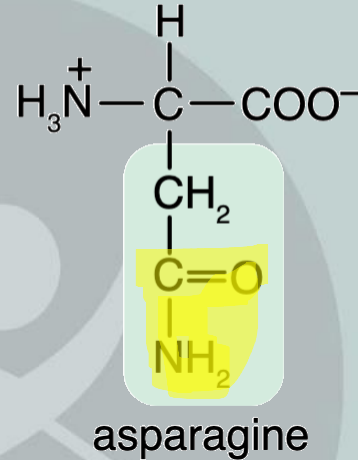
18. Asparagine (N)

N-linked glycosylation

Cold Stress

19. Glutamine (Q)

- Most abundant free amino acid in blood
- major N - transport form from most of tissues to liver

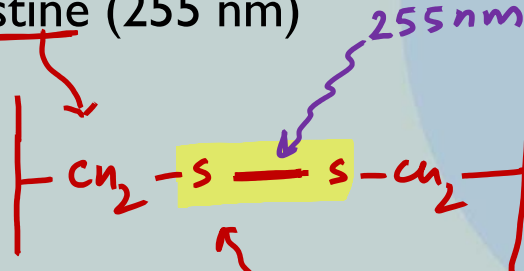




VII. Polar uncharged amino acid with SH group

20. L- Cysteine:

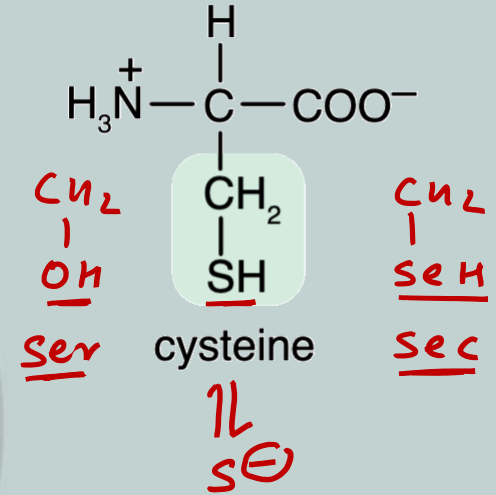
- Exist in R-form
- Cysteine and Methionine Both can be oxidized
- pKa3 value = 8.2
- Ellman's reagent-Free thiol group
- Cystine (255 nm)



Disulphide Bond

oxidizing env^r

Reducing Agent: β -mercapto ethanol (β -me)



Break disulphide Bond

Dithiothreitol (DTT)



21. Selenocysteine (U), sec

- Glutathione Peroxidase
- Formate dehydrogenase

It is incorporated when the mRNA being translated includes a **SECIS element**

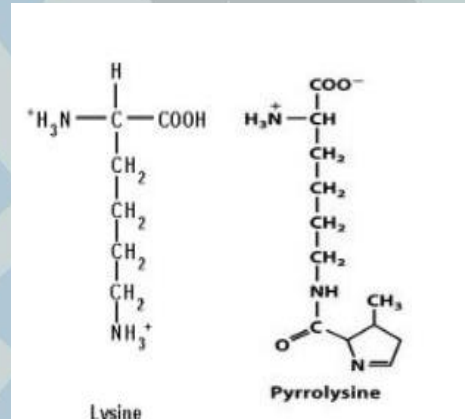
UGA codon encode selenocysteine instead of a stop codon.

22. Pyrrolysine (O), Py1

It is used by some methanogenic archaea

It is coded for with the codon **UAG**,

This UAG codon is followed by a **PYLIS** downstream sequence.





Post Translation Modification

Phosphorylation: Ser, Thr, Tyr, His, Asp

O-linked

Glycosylation: Asn, Ser/Thr

N-linked

Acetylation: Lysine

Hydroxylation

- Proline
- Phenyl Alanine
- Lysine



Glucogenic and ketogenic amino acids:

- Ketogenic: (KL)

- Leu, Lys (Degraded to acetyl-CoA).
Glucose cannot be made from these.

- Glucogenic: (Rest of 14 aa)

Gly, Ala, Val, Pro, Asp, Asn, Glu, Gln, Arg,
His, Ser, Thr, Cys, Met

- ✓ Glucogenic and Ketogenic: (WIFY)

Ile, Phe, Tyr, Trp goes both ways.



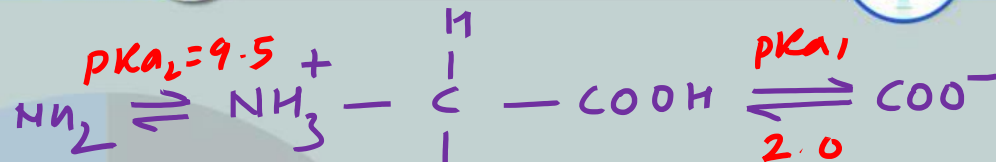
Essential, Semi-essential and non-essential amino acids:

- **Essential:** Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine.
- **Semi essential:** Cysteine, Tyrosine and Arginine
met → Cysteine *phe* → Tyrosine *ornithine* → Arginine
- **Non-essential:** Glycine, Alanine, Serine, Aspartic acid, Asparagine, Glutamic acid, Glutamine, Proline





Amino acid		MW	pK _a ¹	pK _b ²	pK _R ³	pI
Gly	G	75	2.34	9.60	—	5.97
Ala	A	89	2.34	9.69	—	6.00
Val	V	117	2.32	9.62	—	5.96
Leu	L	131	2.36	9.60	—	5.98
Ile	I	131	2.36	9.60	—	6.02
Met	M	149	2.28	9.21	—	5.74
Ser	S	105	2.21	9.15	—	5.68
Thr	T	119	2.09	9.10	—	5.60
Gln	Q	146	2.17	9.13	—	5.65
Asn	N	132	2.02	8.80	—	5.41
Pro	P	115	1.99	10.60	—	6.30
Phe	F	165	1.83	9.13	—	5.48
Trp	W	204	2.83	9.39	—	5.89
Asp	D	133	1.88	9.60	3.65	2.77
Glu	E	147	2.19	9.67	4.25	3.22
Cys	C	121	1.96	10.28	8.18	5.07
Tyr	Y	181	2.20	9.11	10.07	5.66
His	H	155	1.82	9.17	6.00	7.59
Lys	K	146	2.18	8.95	10.53	9.74
Arg	R	174	2.17	9.04	12.48	10.76



α -COOH $pK_{a1} = 2.0$

α -NH₂ $pK_{a2} = 9.5$

R-chain pK_{a3}

Asp 3.65

Glu 4.25

His 6.00

Cys 8.20

Lys 10.5

Arg 12.5



ISO-ELECTRIC POINT : pH where net charge on molecule is zero

✓ **NEUTRAL AMINO ACIDS HAVING TWO pKa VALUES**
pI will be average of 2 pKa values

$$\begin{array}{l} \text{Ala} \quad pI \\ pK_{a1} = 2.0 \\ pK_{a2} = 9.6 \end{array}$$

$$✓ \quad pI = \frac{2.0 + 9.6}{2} = \frac{11.6}{2} = \boxed{5.8}$$

$$\begin{array}{l} pH = pI \\ pH < pI \\ pH > pI \end{array}$$

$$\begin{array}{l} \text{Charge} \\ 0 \\ + \\ - \end{array}$$



pl of Acidic Amino Acids Having 3 pKa Values (Asp, Glu and Cys)

pl will be average of 2 acidic pKa values

PI of Asp

$$pK_{a1} (\alpha\text{-COOH}) = 2.0 \quad \checkmark$$

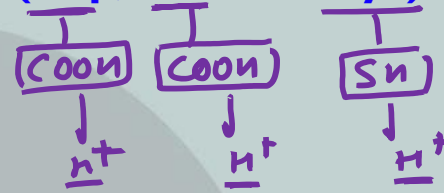
$$pK_{a2} (\alpha\text{-NH}_2) = 9.5$$

$$pK_{a3} (\text{R-COOH}) = 3.6 \quad \checkmark$$

$$pI = \frac{pK_{a1} + pK_{a3}}{2}$$

$$= \frac{2 + 3.6}{2}$$

$$pI = \frac{5.6}{2} = 2.8$$





pI of Basic Amino Acids Having 3 pKa Values (Lys, Arg, His)

pI will be average of 2 basic pKa values

pI of Histidine

$$pK_{a1} (\alpha\text{-COOH}) = 2.0$$

$$pK_{a2} (\alpha\text{-NH}_2) = 9.8$$

$$pK_{a3} (\text{R-imidazole}) = 6.0$$

$$pI = \frac{pK_{a2} + pK_{a3}}{2}$$

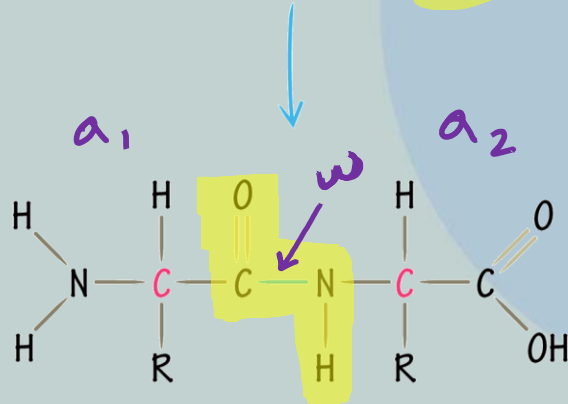
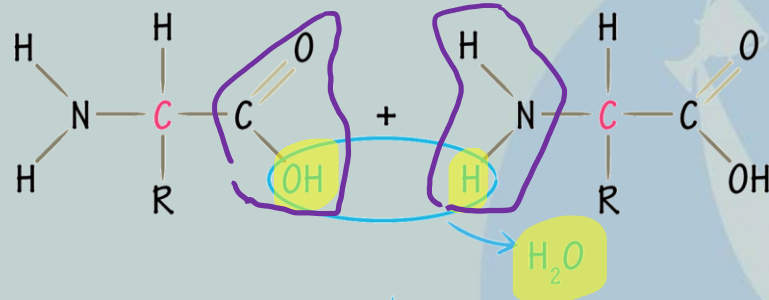
$$= \frac{9.8 + 6.0}{2}$$

$$= \frac{15.8}{2}$$

$$= 7.9$$



Peptide Bond



* Formed betⁿ α -COOH group of aa1 and α -amino group of aa2

* Condensation Rxⁿ
— elimination of water

* Amide bond $\text{—}\overset{\text{O}}{\parallel}\text{C—N—}$
Covalent bond

* rotation is represented by symbol 'w'



✓ The Polypeptide Backbone Is Relatively Polar

✓ Partial double bond character (0.145 nm)

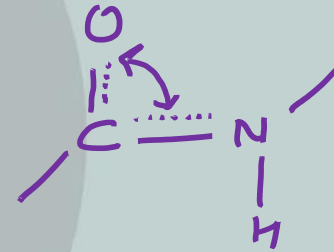
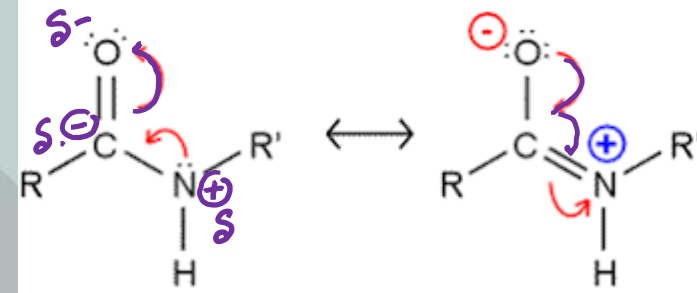


✓ Peptide bond is planar

$C \cdots N$
 ↳ NO rotation allowed
 ↳ ω is fixed = 0° (cis) or 180° (trans)

✓ Shows absorption at 190 nm and 220 nm (Far UV range)

Imp ✓



Trans/Cis peptide bonds

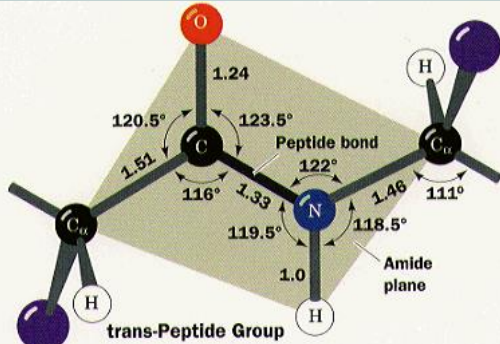


Figure 7-1. The trans-peptide group.

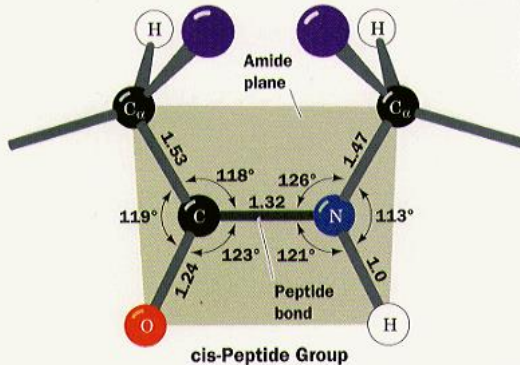
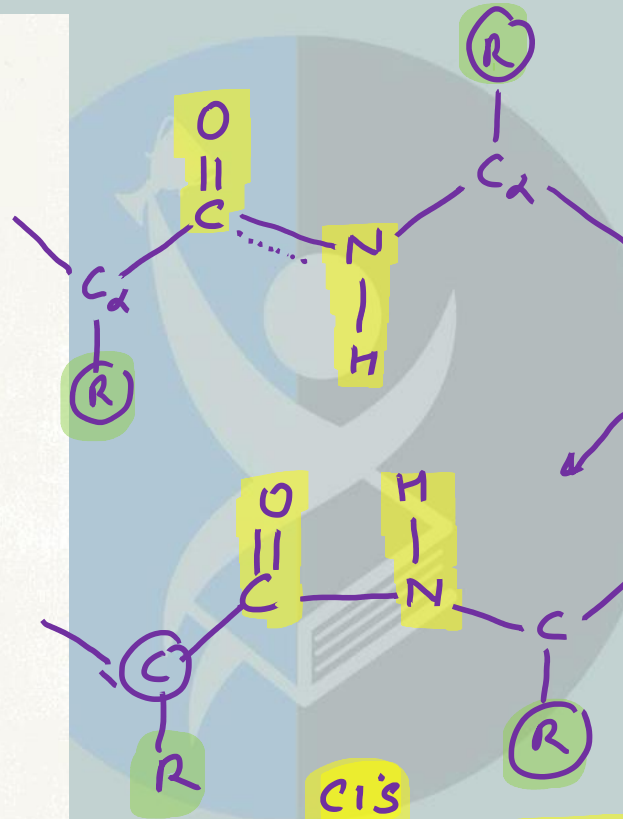


Figure 7-2. The cis-peptide group.



most of aa form
trans peptide bond
Trans $\omega = 180^\circ$

only proline at its
N-terminus can form
cis peptide bond.

$a_1 - a_2 - \text{Pro} - a_3 - a_4$
 \uparrow trans \uparrow cis \uparrow trans \uparrow trans
 $\omega = 0^\circ$

Introduces bent or kink



MW of Peptide

- When particular amino acid is given its specific molecular weight is to be considered, like for **alanine molecular weight is 89 Dalton.**
- Water molecules** would be **eliminated** equal to number of **peptide bonds**

$(Ala)_{10} \rightarrow$ oligopeptide - Linear

$$\begin{aligned} 89 \times 10 &= 890 \\ - 18 \times 9 &= -162 \end{aligned}$$

728(D)

Cyclic

$$\begin{aligned} 89 \times 10 &= 890 \\ - 18 \times 10 &= 180 \end{aligned}$$

710 Dalton

- Average molecular weight of amino acid** when part of protein is **110 Dalton**
- Now no water has to be eliminated**



Apply Your Mind

$$\underline{1 \text{ aa} = 110 \text{ Dalton}}$$

What is the approximate molecular weight of a protein with 100 amino acid residues in a single polypeptide chain?

- ☒ (1) 11 KD
- (2) 15 KD
- (3) 5.2 KD
- (4) 5.5 KD

$$\begin{aligned} & 100 \text{ aa} \\ &= 100 \times 110 \text{ Dalton} \\ &= 11000 \text{ Dalton} \\ &= 11 \text{ KD} \end{aligned}$$

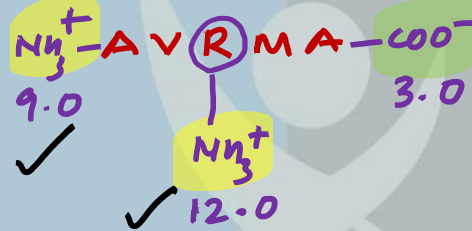


Isoelectric point of polypeptides:

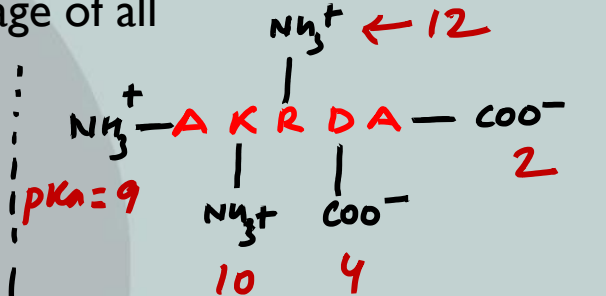
- When there are only 2 pKa value, pI will be average
- When there are 3 pKa value, pI will be average of either two acidic or basic pKa values
- When there are more than 3 pKa value, pI will be average of all



$$\text{pI} = \frac{9+3}{2} = \frac{12}{2} = 6$$



$$\text{pI} = \frac{9+12}{2} = \frac{21}{2} = 10.5$$



$$\text{pI} = \frac{9+10+12+4+2}{5} = \frac{37}{5}$$

$$\text{pI} = 7.4$$



Net charge of peptides at different pH

pH is lower than pKa, there will be protonation

- Acidic group will not bear charge and every basic group will bear +1 charge

$pH < pK_a$

Protonation

- COOH (No charge)
- NH_3^+ (+)

pH is higher than pKa, there will be deprotonation

- Each acidic group will bear charge -1 charge and basic group will bear no charge

9.5



$pH > pK_a$

Deprotonation

- COO^- (-ve)
- NH_2 (No charge)

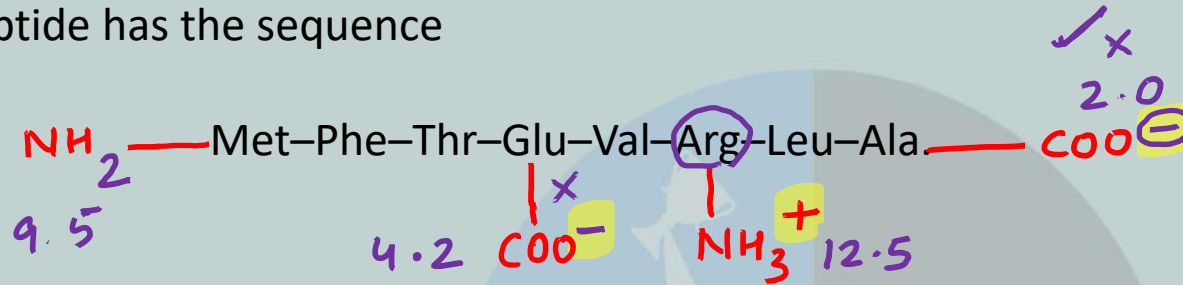
$$\begin{aligned} pH = 1 \quad \text{charge} &= +2 \\ &= +2 \end{aligned}$$

$$\begin{aligned} pH = 7 \quad \text{charge} &= 0 \\ &= 0 \quad pI \end{aligned}$$

$$pH = 12 \quad \text{charge} \rightarrow (-2)$$



A peptide has the sequence



What is the net charge of the molecule at pH 3, 8, and 11?

pH 3

+1

pH 8

-2
+2
0

pH 11

-2
+1
-1



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